

Proteolytic Enzyme Activity of Kentucky and Kansas *Bacillus thuringiensis* Susceptible and Resistant Indianmeal Moths Reared on Transgenic Bt Corn.

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Abstract

Bacillus thuringiensis (Bt) transformed plants are effective for controlling many insect pests, but insect resistance threatens the long term effectiveness of these toxins. Protease-mediated mechanisms may be involved in resistance to Bt and are investigated in this experiment. Enzyme activity data was obtained from Kentucky and Kansas susceptible and resistant Indianmeal moths (IMM), *Plodia interpunctella*, reared on Cry 1F transgenic corn kernels and its non-Bt isolate under laboratory conditions. The Kentucky and Kansas Dipel resistant IMM strains were reared on a 500ppm dipel-treated diet. Eggs of each IMM strain were added to 330 grams of each grain type and were allowed to incubate at 27°C and 60%RH. Emerging adults were collected and their eggs were transferred to new jars of each grain type, and again allowed to incubate. Second generation fourth instar larvae were collected from these jars. The larval guts were excised and tested for enzymatic activity. Microplate assays containing gut extracts, buffer pH 9.2, and the trypsin diagnostic substrate BApNA were run in triplicates. The plates were incubated at 37°C, read at 405nm, and monitored at 30s intervals for 5 min. The mean velocities in units of A/min were calculated. The initial Kentucky and Kansas susceptible strains had statistically higher enzyme activity levels than the Dipel-resistant strains. Both Kentucky and Kansas susceptible strains after two generations of rearing on Cry 1F grain and its non-Bt isolate had significantly reduced enzymatic activity. The Kentucky resistant colony significantly increased its enzymatic activity levels after two generations on both Cry 1F and non-Bt grain. However there was no significant difference between the Kansas resistant strain on either the Cry 1F or its non-Bt isolate after two generations of rearing. The above results show a change in protease activity of Indianmeal moths before and after two generations of rearing on Bt transgenic grain which indicates a possibility for proteases to play a role in resistance.

Introduction

The Indianmeal moth (IMM) is a globally distributed stored grain insect pest. It feeds on a variety of grain and grain products. Its larvae spin silk, damaging grain and grain-handling machinery. It is one of the most abundant moth species infesting stored grain in the Southern United States and is common in Kentucky on-farm stored corn (Sedlacek et al. 1998).

Bacillus thuringiensis (Bt) is a gram-positive, spore-forming bacterium that has been found to be a safe and effective means of control for the Indianmeal moth. IMM are susceptible to Cry 1Aa, 1Ab, 1Ac, 1Ba, 1Ca, 1F, and 2Aa toxins (Schnepf et al. 1998). The IMM was the first species found to develop resistance to Bt in the field (McGaughey 1985). In 1988, McGaughey showed that IMM reared on Bt-treated diet became resistant to *Bacillus thuringiensis* in the laboratory. In addition, Hanley (2001) found laboratory colonies of Kentucky and Kansas IMM became resistant to Cry 1Ab toxin within three generations by 77 and 11 fold, respectively. Finding resistance to Bt at relatively rapid rates is a cause for concern. Bt transgenic corn expresses Bt proteins in kernels and has been found to have a negative impact on IMM in the laboratory (Sedlacek et al. 2001, Giles et al. 2000) and in grain bins (Sedlacek, unpublished data). Cry 1Ab transformed corn kernels caused significant reductions in IMM emergence and fecundity and an increase in duration of development. Cry 1F transformed corn kernels have been found to impact IMM similarly. However, Dipel-resistant colonies do not appear to be cross resistant with Cry 1F kernels.

These findings have led to studies investigating mechanisms of resistance. One proposed mechanism of resistance for IMM is the reduction of gut protease activity (Oppert et al. 1997). The majority of past research has focused on topically applied Bt isolates. Since roughly 20-25% of corn grown in the U.S. is Bt-transformed, researchers also need to look at resistance development and possible mechanisms of resistance to it. Thus the objective of this experiment is to determine the effects of Cry 1F transgenic corn on proteolytic enzyme activity of susceptible and resistant Indianmeal moths.

Materials and Methods

IMM were reared on a ground wheat-based diet. Kentucky and Kansas Bt (Dipel®) resistant IMM strains were reared on 500 ppm Dipel treated diet. Baseline LD₅₀s were obtained from these strains prior to rearing on transgenic grain using purified Cry 1F toxin and a water control. Eggs were added to diet ranging in doses from 0 to 250ppm. Mortality was checked after two weeks and analyzed by Probit analysis.

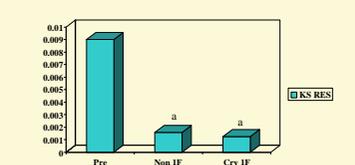
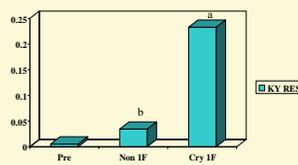
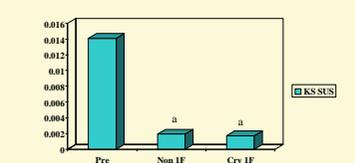
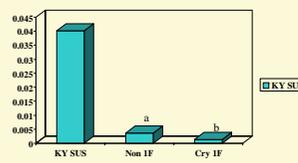
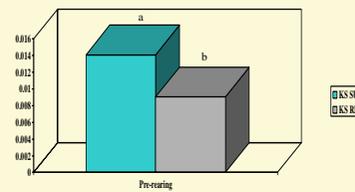
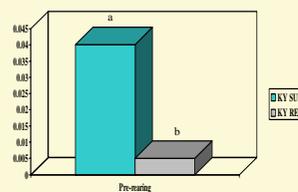
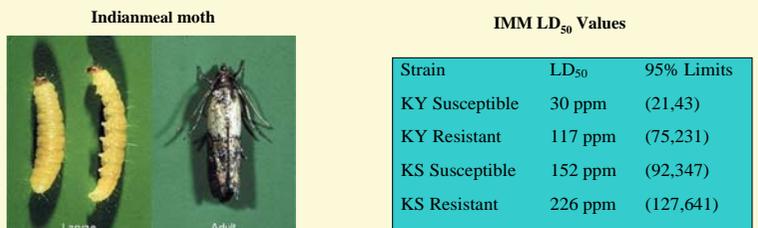
MycoGen 2722 IM1 Cry 1F transformed corn kernels and its non Bt isolate and 7000 eggs from each moth strain were used in this experiment. The experiment consisted of five replications of each treatment for each insect strain. Therefore, one run consisted of 10 jars for each strain. Three hundred and thirty grams of cracked corn were placed in each jar. The corn was cracked for 20 seconds in a standard kitchen blender. The jars were placed in environmental growth chambers at 27±1°C and > 60% relative humidity.

The jars were checked daily after twenty-eight days of incubation. Emerging adults were placed in empty pint jars to lay eggs. Eggs were transferred to new jars containing the same transgenic corn and allowed to incubate again. Late fourth instar larvae were collected from these jars. Their guts were excised and individually submerged in 25 µl of ice-cold dissecting buffer. In microplate rows A-C, 2 µl of gut, 48 µl of Universal buffer (pH 9.2), and 50 µl BApNA substrate were added. Rows D-F contained only 50µl of buffer and 50 µl of BApNA substrate as a control. Plates were incubated at 37°C for one minute, read at 405 nm, and monitored at 30 sec intervals for 5 min. Mean velocities in units of absorbance per minute were calculated. Data was analyzed using analysis of variance (ANOVA) procedures in SAS (1988).

Results and Discussion

Kentucky and Kansas susceptible strains had lower LD₅₀ values compared to the corresponding Dipel-resistant ones. After four generations of rearing on Cry 1F transgenic grain, post LD₅₀ assays will be performed. An increase in the LD₅₀ value will be indicative of resistance development. Also the Kentucky and Kansas Dipel-resistant strains had significantly reduced enzymatic activity compared to the susceptible ones.

The Kentucky susceptible strain reared on Cry 1F corn had significantly reduced enzymatic activity compared to the non-Bt isolate. This data suggests that proteases may play a role in resistance. The Kentucky resistant strain reared on Cry 1F Bt corn had significantly higher enzyme activity compared to those reared on the non-Bt isolate after two generations. There were no changes in enzymatic activity for the Kansas susceptible or resistant IMM reared on Cry 1F Bt corn or its non-Bt isolate after two generations. This data supports findings that populations of moth species from different geographic locations exhibit different susceptibilities/resistance factors to Bt (Tabashnik et al. 1994). However, it is too soon to implicate or rule out the possibility of proteases playing a role in resistance for any IMM strains.



References Cited

- Giles, K.L., R.L. Hellmich, C.T. Iverson, and L.L. Lewis. 2000. Effects of transgenic *Bacillus thuringiensis* maize grain on Bt susceptible *Plodia interpunctella* (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 93: 346-349.
- Hanley, A.M. 2001. Indian meal moth and Angoumois grain moth responses to Cry 1Ab and Cry 9C *Bacillus thuringiensis* transgenic corn and multi cry toxin products and potential for resistance development. MS Thesis, University of Kentucky, Lexington, 109 pp.
- McGaughey, W. 1985. Insect resistance to the biological insecticide *Bacillus thuringiensis*. *Science*, 229(4709): 193-195.
- McGaughey, W. and R.W. Beeman. 1988. Resistance to *Bacillus thuringiensis* in colonies of Indian meal moth and almond moth (Lepidoptera: Pyralidae). *L. Econ. Entomol.* 81(1): 28-33.
- Oppert, B., K.J. Kramer, D. Johnson, and W. McGaughey. 1997. Protease-mediated insect resistance to *Bacillus thuringiensis* toxins. *J. Biol. Chem.* 272: 23477-23480.
- SAS Institute. 1988. SAS/STAT user's guide, release 6.04. SAS Institute. Cary, NC.
- Sedlacek, J.D., P.A. Weston, B.D. Price and P.L. Rattlingourd. 1998. Survey of insect pests in shelled corn on-farm in Kentucky. *J. Entomol. Sci.* 33: 171-179.
- Sedlacek, J.D., S.R. Komaravilli, A.H. Hanley, B.D. Price, and P.M. Davis. 2001. Life History Attributes of Indianmeal moth (Lepidoptera: Pyralidae) and Angoumois grain moth (Lepidoptera: Gelechiidae) reared on transgenic corn kernels. *J. Econ. Entomol.* 94: 586-592.
- Schnepf, E., N. Cickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D.R. Zoegler, and D.H. Dean. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* 62(3): 775-806.
- Tabashnik, B.E., and W. McGaughey. 1994. Resistance risk assessment for single and multiple insecticides: Responses of Indian meal moth (Lepidoptera: Pyralidae) to *Bacillus thuringiensis*. *J. Econ. Entomol.* 87: 834-831.